08/16/04 11/1

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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To:

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EINGEGANGEN 15. Juli 2004

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	13.07.2004

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
E1105-WO	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 02/04153	15.04.2002	08.03.2002

Applicant
EIDGENÖSSISCHE TECHNISCHE HOCHSCHULE LIBRARIES...

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed invention is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Evers, A Tel. +49 89 2399-7061

Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference E1105-WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 02/04153	International filing date *(day/month/year)* 15.04.2002	Priority date *(day/month/year)* 08.03.2002

International Patent Classification (IPC) or both national classification and IPC
G01N33/58

Applicant
EIDGENÖSSISCHE TECHNISCHE HOCHSCHULE LIBRARIES...

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.08.2003	13.07.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Thumb, W Telephone No. +49 89 2399-7350

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 02/04153

I. **Basis of the report**

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-5, 7-38	as originally filed
6, 6a, 6b	received on 09.08.2003 with letter of 07.08.2003

Claims, Numbers

1-31	received on 16.06.2004 with letter of 16.06.2004

Drawings, Sheets

1/10-10/10	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT International application No. PCT/EP 02/04153

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-31
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-31
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-31
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item I

Basis of the report

The claims filed with the letter dated 16.06.2004 appear to contravene the provisions of Article 34(2)b) PCT.
Claims 1, 2, 4, 5, 6, 8, 9, 12, 13, 18 and 21 refer to functional parts of chemical compounds according to the present application, designated b, b', b", b'"; m, m',m",m'"; b1, b1', b1", b1'"; b2, b2', b2", b2'"; and b3, b3', b3", b3'", respectively.
The application as originally filed does not contain a basis for functional parts of molecules designated b", b'"; m",m'"; b1", b1'"; b2", b2'"; or b3", b3'". For example, Figure 2 pertains to a tetramer comprising the chemical moieties p, q, r, s. However, the self-assembly sequences and the identification tags are termed only b1, b1' and b2, b2'. Therefore, the technical features of functional parts designated b", b'"; m",m'"; b1", b1'"; b2", b2'"; or b3", b3'" will not be taken into account in the following report.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Claim 1 meets the requirements of Articles 33(2) and (3) PCT.
   Claim 1 pertains to a combination reaction product of a least two chemical compounds, said compounds comprising a chemical moiety potentially binding to a target molecule and an oligonucleotide, wherein the chemical compounds are bound to each other by self-assembly sequences and the chemical moieties are identified by a unique coding sequence.
   The combination reaction product is stable in the absence of target molecules.
   Document DE-A-196 19 373 (D1), which is considered to represent the most relevant state of the art, discloses libraries of compounds comprising an oligonucleotide part and a binding compound, e.g. a peptidic part, connected via a linking part. Individual molecules can assemble via their nucleic acid part into trimeric structures, thereby assembling the peptidic binding parts to form a binding domain for a substrate (see figure 1). Peptides with different properties from can be assembled and can form a multitude of different combinations compared to individually synthesised peptides (col. 3, lines 21-52). The assembled binding partners can be covalently cross-linked in order to obtain a binding entity for the

target molecule (e.g. col. 4, lines 33-36).
Only in the presence of substrate, supramolecular complexes are formed (col. 3, lines 46-52).
The subject-matter of present claim 1 differs from the teaching of D1 in that the molecules comprise a variable, unique tag for identifying the attached chemical moiety potentially binding to a target molecule and in that the complexes are stable without the substrate being present.
The underlying objective technical problem may therefore be seen in providing an alternative complex of ligands for a target molecule, said ligands being based on the combinatorial formation of supramolecular complexes.
Identifying chemical moieties by using a variable oligonucleotide tag is known in the art (see e.g. documents WO-0023458 (D2, see passages cited in the international search report) and US-A-5 573 905 (D3, abstract)).
However, said documents do not refer to the formation of supramolecular complexes of at least two compounds, thus resulting in spatial proximity of potential target-binding chemical moieties, but only to the identification of individual compounds attached to the tag.
Taking into consideration that D1, besides the lack of identifier tags, explicitly teaches that supramolecular complexes are only formed in the presence of the target molecule, neither document D1 nor D2 and D3, taken alone or in combination, disclose or render obvious a combination reaction product according to present claim 1.
It should further be noted that in the review article by Ramström and Lehn (Nature Reviews, Drug Discovery, Vol. 1(1), pp. 26-36, published two month before the priority date of the present application, an exhaustive list of possible reversible connections is included which can be used in the formation of dynamic combinatorial libraries. However, the use of oligonucleotide self-assembly sequences for this particular purpose is neither disclosed nor even faintly hinted at in this review. Therefore, also from the common general knowledge in the art of dynamic combinatorial libraries, the skilled artisan does not get an indication that would lead to the subject-matter of present claim 1.
Claim 1, as well as dependent claims 2-7, thus meet the requirements of Articles 33(2) and (3) PCT.

2. Independent claims 8 and 18, as well as their respective dependent claims are based on the same principle as the subject-matter of claim 1 and thus also meet

the requirements of Articles 33(2) and (3) PCT.

10/507140
DT15 Rec'd PCT/PTO 07 SEP 2004

Claims

1. A combination reaction product of at least two chemical compounds, each one of these chemical compounds comprising:
   a) a chemical moiety (p,q,r,s) potentially capable of performing a binding interaction with a single target molecule;
   b) an oligonucleotide (b,b',b'',b''') or functional analogue thereof with at least one self-assembly moiety (m,m',m'',m''');

   the chemical compounds being bound to each other by their self-assembly moieties (m,m',m'',m'''), **characterized in that** the combination reaction product is stable in the absence of said target molecule, wherein the oligonucleotides (b,b',b'',b''') or functional analogues of at least one of the chemical compounds comprise a variable, unique coding sequence (b2,b2',b2'',b2''') individually coding for the identification of the particular chemical moiety (p,q,r,s).

2. The combination reaction product of claim 1, **characterized in that** the self-assembly moieties (m,m',m'',m''') are a self-assembly sequences (b1,b1',b1'',b1''') of the oligonucleotides (b,b',b'',b'''), functional analogues thereof, ligands (l) capable to perform a complex reaction with a specific ion (i), or peptides capable of association with other molecules.

3. The combination reaction product of one of claims 1 or 2, **characterized in that** the at least two chemical compounds each comprise a chemical group by which they are covalently linked together after the stable combination reaction product had been formed.

4. The combination reaction product of one of claims 1 to 3, **characterized in that** the oligonucleotides (b,b',b'',b''') or functional analogues thereof are covalently and directly linked to the chemical moieties (p,q,r,s).

5. The combination reaction product of one of claims 1 to 4, **characterized in that** the oligonucleotides (b,b',b'',b''') or functional analogues thereof further comprise a linking portion (b3,b3',b3'',b3''') which is situated between the self-assembly sequence (b1,b1',b1'',b1''') and the chemical moiety (p,q,r,s).

6. The combination reaction product of one of claims 1 to 5, **characterized in that** the coding sequence (b2,b2',b2'',b2''') of oligonucleotide (b,b',b'',b''') or the functional analogue thereof is situated between the chemical moiety (p,q,r,s) and the self-assembly sequence (b1,b1',b1'',b1''').

7. The combination reaction product of one of claims 1 to 6, **characterized in that** it is a dimer, trimer or tetramer exhibiting chemical moieties (p,q,r,s).

8. A chemical library comprising combination reaction products of at least two chemical compounds, each one of these chemical compounds comprising:
   a) a chemical moiety (p,q,r,s) potentially capable of performing a binding interaction with a single target molecule;
   b) an oligonucleotide (b,b',b'',b''') or functional analogue thereof with at least one self-assembly moiety (m,m',m'',m''');

   the chemical compounds being bound to each other by their self-assembly moieties (m,m',m'',m'''), **characterized in that** the combination reaction product is stable in the absence of said target molecule, wherein the oligonucleotides (b,b',b'',b''') or functional analogues of at least one of the chemical compounds comprise a variable, unique coding sequence (b2,b2',b2'',b2''') individually coding for the identification of the particular chemical moiety (p,q,r,s).

9. The chemical library of claim 8, **characterized in that** the self-assembly moieties (m,m',m'',m''') are a self-assembly sequences (b1,b1',b1'',b1''') of the oligonucleotides (b,b',b'',b'''), functional analogues thereof, ligands (l) capable to perform a complex reaction with a specific ion (I), or peptides capable of association with other molecules.

10. The chemical library of one of claims 8 or 9, **characterized in that** the at least two chemical compounds each comprise a chemical group by which they are covalently linked together after the stable combination reaction product had been formed.

11. The chemical library according to one of claims 8 to 10, **characterized in that** it comprises combination reaction products according to any one of claims 4 to 7.

12. The chemical library according to one of claims 8 to 11, **characterized in that** its individual combinations of moieties (p,q,r,s) is derived by forming heteroduplexes, heterotriplexes or heteroquadruplexes of the self-assembly sequences (b1,b1',b1'',b1''') of the oligonucleotides (b,b',b'',b''').

13. The chemical library according to one of claims 8 to 11, **characterized in that** its individual combinations of moieties (p,q,r,s) is derived by chelation of the self-assembly moieties (m,m',m'',m''') with specific ions (I).

14. The chemical library according to claim 12, **characterized in that** it comprises individually encoded sub-libraries (A) and (B), whereas sub-library (A) comprises ***n*** compounds coupled to the 3' extremity of ***n*** different DNA oligonucleotides (b) and sub-library (B) comprises ***m*** compounds coupled to the 5' extremity of ***m*** different DNA oligonucleotides (b').

15. The chemical library according to claim 14, **characterized in that** in sub-library (A) or in sub-library (B) respectively, iodoacetamido- or maleimido-derivatives of ***n*** or ***m*** chemical entities have been coupled to individual DNA oligonucleotides which carry a thiol group at the 3' or 5' end.

16. The chemical library according to claim 14, **characterized in that** in sub-library (A) or in sub-library (B) respectively, amide derivatives - forming chemical structures such as $-O-P(O)_2-O-(CH_2)n-NH-CO-R$, where R may correspond to a number of different chemical entities, and n may range be-

tween 1 and 10 - have been coupled to the oligonucleotides carrying a phosphodiester bond at one extremity.

17. The chemical library according to one of claims 14 to 16, **characterized in that** in sub-library (A) the self-assembly sequence (b1) is interrupted by a d-spacer in opposite position to a code (B), the d-spacer preventing any undesired pairing to the bases of code (B) which encodes sub-library (B), whereas the oligonucleotide (b) of sub-library (A) has its distinctive code (A) towards the 5' extremity.

18. A method of biopanning ligands specific for target molecules, wherein a combination reaction product is incubated with a target molecule, the combination reaction product consisting of at least two chemical compounds, each one of these chemical compounds comprising:

c) a chemical moiety (p,q,r,s) potentially capable of performing a binding interaction with a single target molecule;

d) an oligonucleotide (b,b',b'',b''') or functional analogue thereof with at least one self-assembly moiety (m,m',m'',m''');

wherein the chemical compounds are bound to each other by their self-assembly moieties (m,m',m'',m'''), **characterized in that** the combination reaction product is stable in the absence of said target molecule, wherein the oligonucleotides (b,b',b'',b''') or functional analogues of at least one of the chemical compounds comprise a variable, unique coding sequence (b2,b2',b2'',b2''') individually coding for the identification of the particular chemical moiety (p,q,r,s).

19. The method of claim 18, **characterized in that** combination reaction products according to at least one of claims 1 to 7 are utilized for biopanning.

20. The method of claim 18, **characterized in that** a chemical library of combination reaction products according to at least one of claims 8 to 17 is used for biopanning.

21. A method to identify a target molecule with a combination reaction product comprising a chemical moiety (p,q,r,s) capable of performing a binding interaction with this target molecule and further comprising an oligonucleotide (b,b',b'',b''') or functional analogue thereof, **characterized in that** the combination reaction product is bound to a target by biopanning according to at least one of claims 18 to 20.

22. The method of claim 21, **characterized in that** PCR-fragments are generated by polymerase chain reaction (PCR), each of which carries the code of pairs of sub-library members (A) and (B), whereas sub-library (A) comprises **n** compounds coupled to the 3' extremity of **n** different DNA oligonucleotides (b) and sub-library (B) comprises **m** compounds coupled to the 5' extremity of **m** different DNA oligonucleotides (b').

23. The method of claim 22, **characterized in that** in sub-library (A) or in sub-library (B) respectively, iodoacetamido- or maleido-derivatives of **n** or **m** chemical entities are coupled to individual DNA oligonucleotides, which carry a thiol group at the 3' or 5' end.

24. The method of claim 23, **characterized in that** in sub-library (A) the self-assembly sequence (b1) is interrupted by a d-spacer in opposite position to a code (B), the d-spacer preventing any undesired pairing to the bases of code (B) which encodes sub-library (B), whereas the oligonucleotide (b) of sub-library (A) has its distinctive code (A) towards the 5' extremity.

25. The method of at least one of claims 22 to 24, **characterized in that** the length of the PCR-fragments are checked and their sequence identity is established by digesting the PCR-fragments with a restriction site for a specific endopeptidase (e.g. *EcoRI*), followed by cloning into a suitable plasmid and sequencing.

26. The method of at least one of claims 22 to 25 where several specific binding members are isolated at the end of a biopanning experiment, **character-**

ized in that concatenamers are created, starting from the various PCR-fragments present in the reaction mixture, the concatenated sequences are "read" by sequencing, revealing both the identity and the frequency of pairs of code (A) and code (B).

27. The method of claim 22 where several specific binding members are isolated at the end of a biopanning experiment and sub-libraries (A) and/or (B) carry chemical moieties at the extremities of partially-annealing oligonucleotides **characterized in that** unpaired DNA strands are hybridized with target oligonucleotides (e.g. DNA oligonucleotides) being immobilized on one or more chips.

28. The method of claim 27, **characterized in that** by using chip (A) or chip (B) respectively, the reading of the identity and/or frequency of members of sub-library (A) or sub-library (B) respectively, rescued after a biopanning experiment, is carried out and by decoding on chip (A) and (B) candidate components of sub-libraries (A) and (B), to be re-annealed and screened in a successive round of bio-panning are suggested.

29. The method of claim 28, **characterized in that** increasingly stringent binding to the target is mirrored by a reduction in the number of (A) and/or (B) members as identified on the respective chip and the possible combinations of the candidate (A) and (B) members are assembled individually or in smaller pools and assayed for binding to the target.

30. The method of at least one of the claims 27 to 29, **characterized in that** libraries are allowed to self-assemble in order to form trimeric or tetrameric complexes by using three or four chips, respectively, which carry distinctive target oligonucleotides for decoding.

31. The method of at least one of the claims 27 to 31, **characterized in that** the DNA of selected binding moieties is PCR amplified prior to chip hybridization.